The Patent Office

PCT/GB 9 9 / 0 2 2 4 7

09/743745

INVESTOR IN PEOPLE

82

GB 99/2247

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

REC'D 30 AUG 1999

WIPO PCT

5

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

I also certify that by virtue of an assignment registered under the Patents Act 1977, the application is now proceeding in the name as substituted.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Andrew Gersey

Dated 9 August 1999

GB9815164.0

By virtue of a direction given under Section 30 of the Patents Act 1977, the application is proceeding in the name of

BRAX GROUP LIMITED
Incorporated in the United Kingdom
13 Station Road
CAMBRIDGE
CB1 2JB
United Kingdom [ADP No. 07667546001]

Patents Form 1/77

Act 1977

THE PATENT OFFICE
H
13 JUL 1998
LONDON

The Patent Office

14JUL98 E375407-3 D00068
P01/7700 25.00 - 9815164.0

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

13 JUL 1998

1. Your reference — 87821/JND/CH/kl

2. Patent application number *(The Patent Office will fill in this part)* — **9815164.0**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

BRAX GENOMICS LTD
13 STATION ROAD
CAMBRIDGE CB1 2JB
UNITED KINGDOM

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — UNITED KINGDOM

19587 2000 1.

4. Title of the invention

COMPOUNDS FOR MASS SPECTROMETRY

5. Name of your agent *(if you have one)* — PAGE WHITE & FARRER

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

54 DOUGHTY STREET
LONDON WC1N 2LS

Patents ADP number *(if you know it)* — 1255003

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these appliications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body
See note (d))

YES

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	-
Description	5
Claim(s)	5
Abstract	1
Drawing(s)	1 x (

10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent (Patents Form 7/77)	-
Request for preliminary examination and search (Patents Form 9/77)	-
Request for substantive examination (Patents Form 10/77)	-
Any other documents *(please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Page White & Farrer
Signature
PAGE WHITE & FARRER

Date
13-JULY-1998

12. Name and daytime telephone number of person to contact in the United Kingdom

CHRISTOPHER HILL - 0171 831 7929

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

e) *Once you have filled in the form you must remember to sign and date it.*

f) *For details of the fee and ways to pay please contact the Patent Office.*

COMPOUNDS FOR MASS SPECTROMETRY

This invention concerns compounds which comprise mass markers for detection by mass spectrometry. The invention relates to methods for characterising nucleic acids or other molecules by labelling with markers that are cleavably detachable from their associated nucleic acid and that are detectable by mass spectrometry. Specifically this invention relates to improved methods of detaching mass labels from their associated nucleic acids or other molecules of interest.

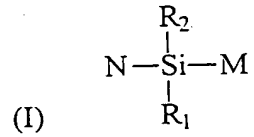
PCT/GB98/00127 describes arrays of cleavable labels that are detectable by mass spectrometry which identify the sequence of a covalently linked nucleic acid probe. These mass labels have a number of advantages over other methods of analysing nucleic acids. At present commercially favoured systems are based on fluorescent labelling of DNA. Fluorescent labelling schemes permit the labelling of a relatively small number of molecules simultaneously, typically 4 labels can be used simultaneously and possibly up to eight. However the costs of the detection apparatus and the difficulties of analysing the resultant signals limit the number of labels that can be used simultaneously in a fluorescence detection scheme. An advantage of using mass labels is the possibility of generating large numbers of labels which have discrete peaks in a mass spectrum allowing similar numbers of distinct molecular species to be labelled simultaneously. Fluorescent dyes are expensive to synthesise whereas mass labels can comprise relatively simple polymers permitting combinatorial synthesis of large numbers of labels at low cost.

A feature of the mass labelling techniques disclosed in PCT/GB98/00127 is the need for linker groups that covalently link a mass marker to its corresponding nucleic acid. These linkers must permit the mass marker to be separated from its nucleic acid prior to detection within a mass spectrometer. It is desirable that the cleavage of the label from its nucleic acid be performed in-line with a mass spectrometer, possibly after some in-line pre-fractionation step such as capillary electrophoresis. It is also desirable that this in-line cleavage step does not require a complex interface with the mass spectrometer to enable this step to occur. Ideally linkers should cleave at some predetermined point within existing instruments without any modification to the instrument beyond changes of normal operating parameters.

Linkers should cleave without damaging associated nucleic acids hence reducing noise in the mass spectrum from nucleic acid fragmentation. Linkers should all cleave under the same conditions to ensure all labels can be analysed simultaneously and quantitatively.

It is an object of this invention to provide linkers that have the desired features disclosed above which are compatible with existing mass spectrometers particularly electrospray ionisation and tandem mass spectrometry.

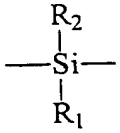
Accordingly, the present invention provides a compound having the following formula (I):



(I)

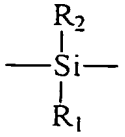
wherein M comprises a mass marker, N comprises a nucleic acid and R_1 and R_2 are substituents selected such that when the compound reacts with an electron donating moiety, either N or M cleaves from the Si atom in preference to R_1 and R_2.

The present invention also provides a method for characterising a nucleic acid or other molecule, which method comprises identifying a mass marker by mass spectrometry, which mass marker is relatable to a specific nucleic acid base or base sequence, or a specific atom or group in a molecule, to identify the mass marker and thereby identify the base or base sequence, or the specific atom or group, wherein the mass marker is attached to the nucleic acid or other molecule by a linker group having the following formula:



wherein R_1 and R_2 are substituents as defined above.

The invention additionally provides use of a linker group having the following formula:



wherein R_1 and R_2 are substituents as defined above, in the characterisation of a nucleic acid or other molecule, by identifying a mass marker by mass spectrometry.

The invention will now be described in further detail by way of example only, with reference to the accompanying drawings, in which:

Figure 1 depicts the mechanism of cleavage of a linker used in the present invention, by means of a primary amine. Cleavage takes place via a five-coordinate intermediate to produce two possible products. The mass spectrum of the charged products is measured.

Compounds with the formula (I) shown above meet the specification disclosed above. The molecule is stable during synthesis and can be cleaved under mild conditions in an electrospray ion source or in the collision chamber of a tandem mass spectrometer in the presence of an appropriately reactive gaseous electron donating moiety, such as ammonia. The reactive gas participates in a novel gas phase reaction with the linker resulting in the cleavage of the linker.

Various substituents may be introduced at the positions R_1 and R_2 including fluorine, chlorine and other halogens, methyl, ethyl and other alkyl groups. Phenyl groups may also be appropriate. Preferably substituents at R_1 and R_2 should be stable during synthesis of the marker, during incorporation of the mass label into an oligonucleotide in an automated synthesiser and under mass spectrometry. It should be apparent to one of ordinary skill in the art that a wide variety of groups will have these properties and may be incorporated into the linker at these positions. It may also be desirable to choose substituents which change the solubility of the linker and alter the rigidity of the linker.

The cleavable linker used in this invention may be cleaved in the ion source of a mass spectrometer by ammonia. However, this invention is not limited to the use of ammonia. Most amines should be capable of separating the mass marker from its cognate oligonucleotide and it is also envisaged that other nucleophiles might be used.

A short alkyl linkage would be appropriate to link the mass marker to the linker although it should be apparent to one of ordinary skill in the art that a wide variety of linkages are available which can be used to link a mass marker to a linker.

Mass markers appropriate for use with this invention are disclosed in PCT/GB98/00127 and the co-pending UK application, of Page White and Farrer file number 87820. PCT/GB98/00127 discloses the use of substituted poly-ethers. These are highly favoured for use as mass markers with this invention. Co-pending application in PWF file 87820 discloses mass labels which chelate metals ions and are thus pre-ionised before mass spectrometry. These labels have a higher sensitivity and signal to noise ratio than other mass markers. These are also highly favoured mass markers for use with this invention.

It should be noted that this invention is not limited to the mass markers disclosed in the above applications. Any molecule with the correct features can be used as a mass marker. Desirable features include:

Easily detachable from DNA
Fragmentation resistant in mass spectrometer
Single Ion Peaks
Very sensitive detection
Easily distinguishable from background contamination
Distinguish from DNA
Be certain that a mass peak is from a mass label
Compatible with oligonucleotide synthesiser

Easy to synthesise in a combinatorial manner to minimise number of chemical steps and the number of reagents necessary to generate large number of labels

Compatible with existing mass spectrometry instrumentation without requiring physical modification.

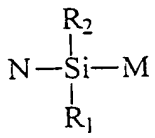
Mass labels and their linkers can be attached to a nucleic acid molecule at a number of locations in the nucleic acid. For conventional solid phase synthesisers the 5' hydroxyl of the sugar is the most readily accessible. Other favoured positions for modifications are on the base at the 5' position in the pyrimidines and the 7' and 8' positions in the purines. These would all be appropriate positions to attach a cleavable mass with the linker of this invention.

The 2' position on the sugar is accessible for mass modifications but is more appropriate for small mass modifications that are not to be removed.

The phosphate linkage in natural nucleic acids can be modified to a considerable degree as well, including derivitisation with mass labels.

Claims:

1. A compound having the following formula:



wherein M comprises a mass marker, N comprises a nucleic acid and R_1 and R_2 are substituents selected such that when the compound reacts with an electron donating moiety, either N or M cleaves from the Si atom in preference to R_1 and R_2.

2. A compound according to claim 1, wherein R_1 and R_2 are selected such that their bond energies to Si are greater than the bond energy of N and/or M to Si to ensure that when the compound is reacted with an electron donating moiety either N or M cleaves from the Si atom in preference to R_1 and R_2, and/or R_1 and R_2 are selected such that their steric bulk is sufficient to ensure that when the compound is reacted with an electron donating moiety either N or M cleaves from the Si atom in preference to R_1 and R_2.

3. A compound according to claim 1 or claim 2, wherein R_1 and R_2 are each independently a hydrogen atom, a halogen atom, a substituted or unsubstituted alkyl group, or a substituted or unsubstituted aryl group.

4. A compound according to claim 3, wherein R_1 and R_2 are each independently fluorine, chlorine, bromine, iodine, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl or phenyl groups.

5. A compound according to any preceding claim, wherein N comprises a nucleotide or an oligonucleotide.

6. A compound according to claim 5, wherein the nucleotide or oligonucleotide is natural, or is modified by modifying a base, sugar and/or backbone of the nucleotide or oligonucleotide.

7. A compound according to any preceding claim, wherein the mass marker comprises a polyether.

8. A compound according to claim 7, wherein the polyether is a substituted or unsubstituted poly(arylether).

9. A compound according to claim 7 or claim 8, wherein the polyether comprises one or more fluorine atom substituents.

10. A compound according to any preceding claim, wherein the mass marker comprises a metal ion-binding moiety.

11. A compound according to claim 10, wherein the metal ion-binding moiety is a porphyrin, a crown ether, hexahistidine, or a multidentate ligand.

12. A compound according to claim 11, wherein the metal ion-binding moiety is a bidentate ligand or is EDTA.

13. A compound according to any of claims 10-12, wherein the metal ion-binding moiety is bound to a monovalent, divalent or trivalent metal ion.

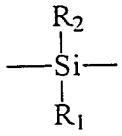
14. A compound according to claim 13, wherein the metal ion is a transition metal ion, or a metal ion of group IA, IIA or IIIA of the periodic table.

15. A compound according to claim 14, wherein the metal ion is Ni^{2+}, Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, or Al^{3+}.

16. A compound according to any preceding claim, wherein the electron donating moiety is a Lewis base.

17. A compound according to claim 16, wherein the Lewis base is ammonia; a primary, secondary or tertiary amine; a compound containing a hydroxy group; an ether; or water.

18. A method for characterising a nucleic acid or other molecule, which method comprises identifying a mass marker by mass spectrometry, which mass marker is relatable to a specific nucleic acid base or base sequence, or a specific atom or group in a molecule, to identify the mass marker and thereby identify the base or base sequence, or the specific atom or group, wherein the mass marker is attached to the nucleic acid or other molecule by a linker group having the following formula:



wherein R_1 and R_2 are substituents as defined in claim 1.

19. A method according to claim 18, wherein R_1 and R_2 are substituents as defined in any of claims 2-4.

20. A method according to claim 18 or claim 19, wherein the mass marker is as defined in any of claims 7-9.

21. A method according to any of claims 18-20, wherein the mass marker comprises a metal ion-binding moiety.

22. A method according to claim 21, wherein the metal ion-binding moiety is as defined in claim 11 or claim 12.

23. A method according to claim 21 or claim 22, which method further comprises binding a metal ion as defined in any of claims 13-15 to the metal ion-binding moiety, prior to identifying the mass marker by mass spectrometry.

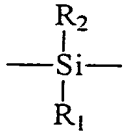
24. A method according to any of claims 18-23, which method further comprises forming a compound as defined in any of claims 1-17, prior to identifying the mass marker.

25. A method according to any of claims 18-24, which method further comprises contacting the linker group with an electron donating moiety to cleave off the mass marker.

26. A method according to claim 25, wherein the electron donating moiety is as defined in claim 16 or claim 17.

27. A method according to any of claims 18-26, which method further comprises cleaving off the mass marker in the mass spectrometer.

28. Use of a linker group having the following formula:



wherein R_1 and R_2 are substituents as defined in any of claims 1-4, 16 and 17 in the characterisation of a nucleic acid or other molecule, by identifying a mass marker by mass spectrometry.

29. Use according to claim 28, wherein the nucleic acid or other molecule is as defined in claim 5 or claim 6.

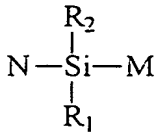
30. Use according to claim 28 or claim 29, wherein the mass marker is as defined in any of claims 7-15.

31. Use according to any of claims 28-30, wherein the mass marker forms part of a compound as defined in any of claims 1-17.

ABSTRACT

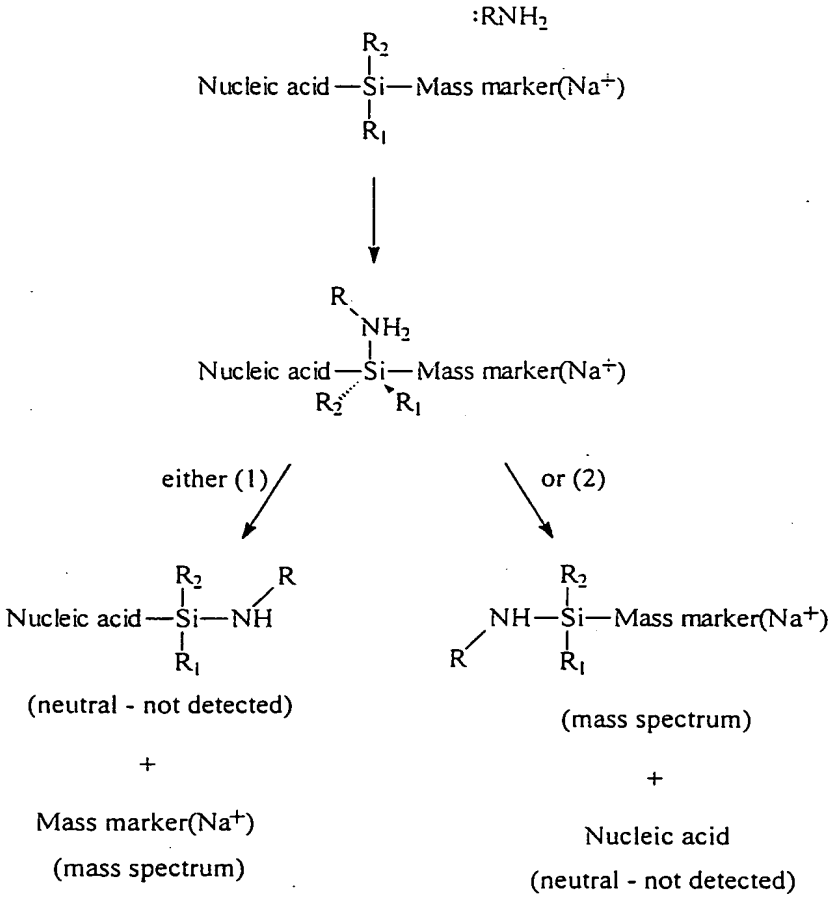
COMPOUNDS FOR MASS SPECTROMETRY

Provided is a compound having the following formula:



wherein M comprises a mass marker, N comprises a nucleic acid and R_1 and R_2 are substituents selected such that when the compound reacts with an electron donating moiety, either N or M cleaves from the Si atom in preference to R_1 and R_2.

Figure 1


:RNH2
R2
Nucleic acid—Si—Mass marker(Na+)
R1
R
NH2
Nucleic acid—Si—Mass marker(Na+)
R2
R1
either (1)
or (2)
R2
R
Nucleic acid—Si—NH
R1
(neutral - not detected)
+
Mass marker(Na+)
(mass spectrum)
R2
NH—Si—Mass marker(Na+)
R
R1
(mass spectrum)
+
Nucleic acid
(neutral - not detected)

PCT/GB99/02247

13.7.99

Page White & Farrer

98151164.0